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Nutritional requirements for the production of antimicrobial metabolites from *Streptomyces*

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The objective of this study was to optimize the nutritional and cultural conditions of *Streptomyces* strain ERI-1, ERI-3 and ERI-26 for the production of antimicrobial metabolites under shake-flask conditions. Effect of eight fermentation medium, different temperature, pH, incubation time, different carbon and nitrogen sources and different concentration of sodium chloride on production of antimicrobial metabolites were studied. Antimicrobial activity of the fermentation medium was evaluated by cup plate method by measuring the zone of inhibition. Nutritional and cultural conditions for the production of antimicrobial metabolites by *Streptomyces* strain ERI-1, ERI-3 and ERI-26 under shake-flask conditions have been optimized. Modified nutrient medium was found to be good base for fermentation. Glucose and ammonium nitrate were identified as best carbon and nitrogen sources, respectively for growth and production of more antimicrobial compounds. Similarly, initial production medium pH of 7.0, incubation temperature of 30°C and incubation time of 96 h was found to be optimal. Optimization of medium and cultural conditions resulted in better antibacterial and antifungal activity. The zone of inhibition of ERI-26 against *Aspergillus niger* was 25 and 20 mm for *Curvularia lunata*, respectively. It is clear that novel *Stretomyces* strains ERI-1, ERI-3 and ERI-26 produced extra cellular antimicrobial metabolites effective against pathogenic bacteria and fungi, moreover the medium and cultural conditions for better antimicrobial metabolites production have been optimized.

Key words: *Streptomyces*, antimicrobial activity, nutritional requirements, cultural conditions, optimized media.

INTRODUCTION

Streptomycetes are potent producers of secondary metabolites. Among 10000 known antibiotics, 45-55% is produced by Streptomycetes (Demain, 2006; Lazzarini et al., 2000). Secondary metabolites produced by them have a broad spectrum of biological activities such as antibacterial (streptomycin, tetracycline, chloramphenicol),

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antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin), immunosuppressive (rapamycin), anticancer (actinomycin, mitomycin C, anthracyclines), enzyme inhibitors (clavulanic acid) and diabetogenic (bafilomycin, streptozotocin).

Secondary metabolite production in microbes is strongly influenced by nutritional factors and growth conditions. A common strategy, widely applied in industrial screening programs, consists of the application of several growth conditions (including variables such as production media, incubation time and other factors) to different strains which has been studied (Vilella et al., 2000). There is strong evidence that this approach improves the chances of finding the desired metabolites (Lauren and Yit-Heng, 2011; Arasu et al., 2013). Considering that any screening program of microbial natural products can handle a limited number of fermentations, applying many growth conditions to each strain may critically restrict the number of strains and therefore the diversity of biosynthetic pathways included in the screening. As a compromise between these two requirements, a number of conditions are usually applied to each strain (Wildmans, 1997). Furthermore, without prior knowledge about the preferred growth conditions for a given microorganism, random assignment of media to strains may generate an inefficient redundancy of metabolites or extracts lacking relevant levels of secondary metabolites. The production of secondary metabolites is affected by the availability of nutrients (Valanarasu et al., 2010). In fermentation experiments, the production of antibiotics is increased by the presence of a non preferred carbon source, or by other nutrients (Mohd et al., 2012). The source and availability of nitrogen can also influence the production of secondary metabolites (Arasu et al., 2008; Arasu et al., 2009).

In our screening programme for isolation and identification of actinomycetes from Westen Ghats of Tamil Nadu for antimicrobial metabolite production, we isolated 367 actinomycetes from different parts of the forest soil samples. Actinomycetes recovered from Kanyakumari showed significant activity against most of the tested bacteria and fungi (Arasu et al., 2013). Among the different actinomycetes; ERI-1, ERI-3 and ERI-26 isolates from the Western Ghats region of Kanyakumari District revealed significant activity against all the tested bacteria and fungi. This study focused on optimizing nutrition and cultural conditions of Streptomyces strain ERI-1, ERI-3 and ERI-26 for the production of antimicrobial metabolites under the shake-flask condition.

MATERIALS AND METHODS

Fermentation medium

Fermentations were carried out using eight different antimicrobial compound production medium; GEN medium (M-1), *Streptomyces* medium (M-2), *Micromonospora* medium (M-3), nutrient glucose medium (M-4), Bennett medium (M-5), starch with a mineral salt solution medium (M-6), complex medium (M-7) and starch casein medium (M-8). A loopful of a selected strain was inoculated into 50 mL fermentation broth medium and incubated on a rotary shaker at 200 rpm, 30°C for 24 h. After that 10% of inoculum was transferred to production medium containing 100 mL of fermentation medium in 500 mL Erlenmeyer flask. Different culture conditions like temperatures (20, 25, 30, 37 and 45°C), pH (6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) and incubation time (24, 48, 72, 96, 120 and 144 h) were studied to standardize the antibiotic production. Effect of different carbon source and nitrogen source for growth and antimicrobial compound production in fermentation medium were evaluated. Different concentrations of sugar for production of antimicrobial compound and growth were also studied. Influence of different concentrations of sodium chloride in production medium was studied.

Antibacterial assay

Test organism

The following test organisms were used for antibacterial studies: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (MTCC 3615), *Bacillus subtilis* (MTCC 441), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 15380), *Proteus vulgaris* (MTCC 1771), *Erwinia* sp. MTCC (2760), *Xanthomonas* sp., *Vibrio fischeri* (MTCC 1738) and *Salmonella typhi* (MTCC 733). All cultures were obtained from Department of Microbiology, Christian Medical College, Vellore, Tamil Nadu, India. Each bacterial strain was inoculated in 3 mL of Mueller Hinton broth and incubated at 37°C for 24 h. After incubation period the culture was diluted.

Fungal strains

The following fungi were used for experiments: *Trichophyton rubrum* (MTCC 296), *Trichophyton rubrum* (57/01), *Trichophyton mentagrophytes* (66/01), *Trichophyton simii* (110/02), *Epidermophyton floccosum* (73/01), *Scopulariopsis* sp. (101/01) *Aspergillus niger* (MTCC 1344), *Botyritis cinerea*, *Curvularia lunata* (46/01) and *Candida albicans* (MTCC 227).

Preparation of fungal spore

The filamentous fungi were grown on Sabouraud dextrose sgar (SDA) slants at 28°C for 10 days and the spores were collected using sterile doubled distilled water and homogenized. Yeast was grown on Sabouraud dextrose broth at 28°C for 48 h.

Cup plate method

Antibacterial assays

Antibacterial activities were assayed by the agar diffusion method by Arasu et al. (2013). Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures (100 µL) of suspension containing 10^8 CFU/mL bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. 5 mm diameter well was made using sterile cork borer and filled with 0.05 mL of supernatant of ERI-1, ERI-3 and ERI-26. The plates were left for 30 min at room temperature for supernatant diffusion. Negative control was prepared using the respective fermentation medium. The plates were incubated for 24 h at 37°C. A zone of inhibition was recorded in millimeters and the experiment was repeated twice.

Antifungal assays

Antifungal assay was done by agar diffusion method. Petri plates

Table 1. Effect of production of antimicrobial metabolites on different media.

Fermentation medium	Inhibition zones in mm												
	B.s	***S.a***	***S.e***	***E.f***	***E.c***	***P.a***	***K.p***	***X.*sp.**	***E.*sp.**	***S.t***	***V.f***	***P.v***	***C.a***
ERI-1													
Medium -1	12	10	10	14	10	10	9	9	12	10	10	13	10
Medium – 2	10	12	12	12	10	10	-	-	-	-	-	-	-
Medium – 3	12	10	23	10	11	10	10	-	-	-	-	-	-
Medium – 4	14	12	14	15	14	11	10	10	12	10	10	10	14
Medium – 5	10	12	15	-	-	-	-	-	-	-	-	-	10
Medium – 6	12	13	10	12	10	10	10	12	12	11	10	-	-
Medium – 7	10	14	-	-	-	-	-	-	-	-	-	-	-
Medium – 8	12	12	10	10	10	10	12	11	12	10	9	9	12
ERI-3													
Medium - 1	10	12	13	10	10	10	9	9	10	-	-	-	-
Medium – 2	12	11	12	10	11	10	11	10	10	-	-	-	-
Medium – 3	10	9	11	10	-	-	-	-	-	-	-	-	10
Medium – 4	10	10	9	9	9	12	-	-	-	-	-	-	-
Medium – 5	15	12	10	-	-	-	-	-	-	-	-	-	10
Medium – 6	11	10	11	11	10	10	12	10	10	-	-	-	-
Medium – 7	11	-	-	-	-	-	-	-	-	-	-	-	-
Medium – 8	12	14	14	10	12	11	10	12	15	10	12	10	12
ERI-26													
Medium – 1	9	9	11	10	-	-	-	-	-	-	-	-	10
Medium – 2	11	13	14	10	11	9	9	10	-	-	-	-	-
Medium – 3	10	11	12	10	-	-	-	-	-	-	-	-	-
Medium – 4	12	14	14	12	15	10	11	12	12	10	14	10	10
Medium – 5	11	12	12	10	-	-	-	-	-	-	-	-	10
Medium – 6	11	10	10	-	-	-	-	-	-	-	-	-	-
Medium – 7	10	-	-	-	-	-	-	-	-	-	-	-	-
Medium – 8	9	11	10	10	9	-	-	-	-	-	-	-	10

-: No activity, ERI: Entomology Research Institute. *B.s- B. subtilis*; *S.a- S. aureus; S.e-S. epidermidis; E.f- E. faecalis; E.c-E. coli*; *P.a- P. aeruginosa; K.p- K. pneumoniae; X.sp- Xanthomonas* sp.; *E.sp- Erwinia; S.t- S. typhi*; *V.f- V. fischer*i; *P.v- P. vulgaris.* Results were analyzed in triplicates.

were prepared with 20 mL of sterile SDA (Hi-media, Mumbai). The test cultures (50µL of fungal spore suspension) were swabbed on the top of the solidified media and allowed to dry for 10 min. Five mm diameter well was made using sterile cork borer and filled with 0.05 mL of supernatant of ERI-1, ERI-3 and ERI-26. The plates were left for 30 min at room temperature for supernatant diffusion. Negative control was prepared using the respective fermentation medium. The plates were incubated for 24-48 h at 28°C. A zone of inhibition was recorded in millimeters and the experiment was repeated twice.

RESULTS

Selection of fermentation medium

Eight different fermentation media named as M-1 to M-8 were used as the base to determine the optimal nutritional conditions for the production of antimicrobial compound(s) from three actinomycetes strains ERI-1, ERI-3 and ERI-26. All the twelve tested bacteria and yeast growth was inhibited by strain ERI-1 grown in M-1 and M-8 medium. M-2 did not show any activity against *K. pneumoniae, Xanthomonas* sp., *Erwinia, S. typhi, V. fischeri*, *P. vulgaris* and *C. albicans,* whereas M-5 exhibited activity against only *B. subtilis, S. aureus* and *S. epidermidis* (Table 1). ERI-3 cultivated in M-3, M-5 and M-8 inhibited the growth of all the tested bacteria; however M-4 was ideal for ERI-26

Effect of pH on the production of antimicrobial compounds

Optimum pH for the production of antimicrobial compounds was recorded by diameter of zone of inhibition (Table 2). At pH 7.0, ERI-1, ERI-3 and ERI-26 exhibited

Table 2. Effect of pH of the medium on the growth and production of antimicrobial metabolites.

pH	Strain	Inhibition zones in mm												
		B.s	***S.a***	***S.e***	***E.f***	***E.c***	***P.a***	***K.p***	***X.sp***	***E.sp***	***S.t***	***V.f***	***P.v***	***C.a***
6.0	ERI-1 DCW 1.5g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-3 DCW 0.98 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-26 DCW 1.23 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
6.5	ERI-1 DCW 1.74 g/L	12	9	11	10	12	-	-	-	-	-	-	-	10
	ERI-3 DCW 1.32g/L	9	10	10	11	9	9	9	-	-	-	-	-	-
	ERI-26 DCW 1.47 g/L	10	11	12	10	10	9	7	-	-	-	-	-	-
7.0	ERI-1 DCW 2.47 g/L	15	15	14	14	14	11	10	10	12	12	12	10	12
	ERI-3 DCW 2.21 g/L	12	9	9	11	10	9	9	9	11	-	-	-	-
	ERI-26 DCW 2.73 g/L	13	13	16	16	13	10	11	12	12	11	13	10	12
7.5	ERI-1 DCW 2.4 g/L	9	10	10	9	9	10	9	10	12	10	9	9	9
	ERI-3 DCW 2.75 g/L	12	13	15	10	11	11	10	12	14	10	12	10	11
	ERI-26 DCW 2.43 g/L	9	10	9	9	10	8	8	12	13	14	-	-	10
8.0	ERI-1 DCW 1.41 g/L	13	14	10	12	12	14	12	-	-	-	-	-	-
	ERI-3 DCW 1.28 g/L	9	10	11	9	9	9	-	-	-	-	-	-	12
	ERI-26 DCW 0.79 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
8.5	ERI-1 DCW 0.42 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-3 DCW 0.61 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-26 DCW 0.72 g/L	-	-	-	-	-	-	-	-	-	-	-	-	-

-; No activity, ERI- Entomology Research Institute, DCW- dry cell weight. *B.s - B. subtilis; S.a – S. aureus ;S.e - S. epidermidis; E.f - E. faecalis; E.c - E. coli*; *P.a- P. aeruginosa; K.p - K. pneumoniae; X.sp - Xanthomonas* sp.; *E.sp- Erwinia;S.t - S. typhi*; *V.f - V. fischeri*;*P.v - P. vulgaris.* Results were analyzed in triplicates.

good growth and antimicrobial activity against tested bacteria. Cell growth in terms of biomass was noted in strain ERI-3 as 0.98, 1.32, 2.21, 2.43, 0.79 and 0.72 g/L for pH 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5, respectively.

Optimizing the incubation time and temperature for production of antimicrobial compounds

ERI-1, ERI- 3 and ERI- 26 showed maximum antimicro-

Table 3. Effect of incubation time on growth and production of antimicrobial metabolites.

Time	Strain	Inhibition zones in mm												
		B.s	***S.a***	***S.e***	***E.f***	***E.c***	***P.a***	***K.p***	***X.sp***	***E.sp***	***S.t***	***V.f***	***P.v***	***C.a***
24	ERI-1 DCW 1.27 (g/L)	9	9	10	-	-	-	-	-	-	-	-	-	-
	ERI-3 DCW 0.7 (g/L)	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-26 DCW 0.86 (g/L)	-	-	-	-	-	-	-	-	-	-	-	-	-
48	ERI-1 DCW 2.8	10	12	10	9	11	12	13	11	12	10	12	12	10
	ERI-3 DCW 2.46 (g/L)	9	10	12	9	10	11	10	10	12	11	10	10	10
	ERI-26 DCW 2.1 (g/L)	10	9	11	12	9	10	11	9	11	10	10	10	9
72	ERI-1 DCW 3.21 (g/L)	9	10	11	10	11	12	12	10	12	11	11	10	10
	ERI-3 DCW 4.01 (g/L)	12	11	12	10	11	13	10	9	12	10	12	10	10
	ERI-26 DCW 3.9 (g/L)	10	12	11	12	13	10	11	12	12	10	13	10	10
96	ERI-1 DCW 3.02 (g/L)	9	10	9	9	10	9	10	10	9	10	9	10	10
	ERI-3 DCW 3.79 (g/L)	12	10	11	11	10	11	9	10	10	9	11	10	10
	ERI-26 DCW 4.0 (g/L)	10	9	11	10	10	9	11	12	9	10	10	10	9

-; No activity, ERI- Entomology Research Institute, DCW- dry cell weight; *B.s - B. subtilis; S.a- S. aureus; S.e- S. epidermidis; E.f - E. faecalis; E.c - E. coli*; *P.a- P. aeruginosa; K.p - K. pneumoniae; X.sp - Xanthomonas* sp.; *E.sp- Erwinia; S.t- S. typhi*; *V.f - V. fischer*i; *P.v - P. vulgaris.* Results were analyzed in triplicates.

bial activity after 24 h. At 48 and 72 h, it exhibited maximum cell growth as well as antibacterial activity (Tables 3 and 4). ERI-1, ERI-3 and ERI-26 did not exhibit activity at 20 and 45°C. It was not suitable for growth also. 30°C was found to be optimum temperature for antimicrobial metabolite production (Table 4).

Effect of different carbon sources on growth and antimicrobial activity

Effect of different carbon source on growth and antimicrobial activity was analyzed. Optimization of antibacterial compound production was carried out in batch culture. Strains were able to grow in all the tested carbon sources (Table 5). However, ERI-1 and ERI-3 exhibited maximum antimicrobial activity in medium supplemented with glucose as a sole carbon source followed by starch and fructose. ERI-26 was able to utilize glucose for better growth and revealed maximum antibacterial activity.

Effect of glucose concentration on growth and antimicrobial activity

Different concentration of glucose on growth and antibacterial activity was studied (Table 6). For ERI-1, ERI-3 and ERI-26, the concentration of glucose from 6 to 12 g/L enhanced the cell growth and the antimicrobial compound production.

Effect of different nitrogen source on growth and antimicrobial activity

The results of nitrogen source utilization are shown in Table 7. The higher growth and antibacterial activity were observed in ammonium nitrate as nitrogen source followed by sodium nitrate and potassium nitrate in case of ERI-1 and ERI-26. ERI-3 exhibited maximum growth and antibacterial activity by using sodium nitrate followed by ammonium nitrate.

Table 4. Effect of incubation temperature on growth and production of antimicrobial metabolites.

Temperature	Strain	Inhibition zones in mm												
		B.s	***S.a***	***S.e***	***E.f***	***E.c***	***P.a***	***K.p***	***X.sp***	***E.sp***	***S.t***	***V.f***	***P.v***	***C.a***
20°C	ERI-1	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-3	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-26	-	-	-	-	-	-	-	-	-	-	-	-	-
30°C	ERI-1	10	11	11	14	10	12	10	11	10	10	10	11	10
	ERI-3	11	9	11	13	10	13	11	12	10	10	10	10	12
	ERI-26	9	11	12	10	9	10	10	10	11	11	11	10	11
37°C	ERI-1	10	10	9	11	9	11	10	9	9	-	-	-	9
	ERI-3	9	10	10	9	9	-	-	9	9	9	10	11	10
	ERI-26	9	11	10	9	9	9	10	10	10	10	10	9	9
45°C	ERI-1	10	11	-	-	-	-	-	-	-	-	-	-	-
	ERI-3	-	-	-	-	-	-	-	-	-	-	-	-	-
	ERI-26	-	-	-	-	-	-	-	-	-	-	-	-	-

-: No activity, ERI- Entomology Research Institute. *B.s- B. subtilis*; *S.a- S. aureus; S.e- S. epidermidis*; *E.f- E. faecalis*; *E.c- E. coli*; *P.a- P. aeruginosa*; *K.p- K. pneumoniae*; *X.sp- Xanthomonas* sp.; *E.sp- Erwinia*; *S.t- S. typhi*; *V.f- V. fischer*i; *P.v- P. vulgaris*.Results were analyzed in triplicates.

Table 5. Effect of different carbon sources on growth and antimicrobial activity against *S. epidermidis*.

Carbon source	ERI-1		ERI-3		ERI-26	
	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)
Glucose	2.9	23	3.1	20	3	23
Starch	1.7	20	1.5	18	1.7	17
Sucrose	1.8	17	1.7	15	2	16
Starch	1.5	20	1.4	15	1.8	16
Maltose	2.7	18	2.9	17	2.5	18
Fructose	2.8	20	2.7	19	2.9	19
Mannitol	0.8	15	0.4	15	0.5	16
Mannose	1.7	15	1.5	15	1.8	15
Xylose	0.6	0	0.7	13	0.4	-
Glycerol	2.4	18	2.7	16	2.9	16

Results were analyzed in triplicates.

Table 6. Effect of different glucose concentration on growth and antimicrobial activity (against *S. epidermidis*).

Glucose concentration (g/L)	ERI-1		ERI-3		ERI-26	
	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)
2	0.72	-	0.45	-	0.91	12
4	1.25	-	0.93	-	1.43	12
6	2.37	14	1.73	16	2.30	15
8	3.11	17	2.54	17	3.10	17
10	3.73	17	3.1	19	3.80	20
12	3.68	22	3.04	19	3.90	22
14	3.24	22	2.73	18	2.40	22
16	2.43	20	2.64	18	2.34	22
18	1.40	20	1.56	14	2.10	18
20	0.91	18	1.24	14	2.03	18

Results were analyzed in triplicates.

Table 7. Effect of different nitrogen sources on growth and antimicrobial activity (against *S. epidermidis*).

Nitrogen source	ERI-1		ERI-3		ERI-26	
	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)
Ammonium nitrate	2.78	21	2.42	17	2.40	17
Ammonium sulphate	0.87	14	1.27	15	1.70	15
Ammonium citrate	1.04	15	0.73	12	0.64	-
Sodium nitrate	2.45	20	2.75	22	1.92	15
Potassium nitrate	2.40	19	2.35	17	2.29	15

Results were analyzed in triplicates.

Table 8. Effect sodium chloride concentration on growth and antimicrobial activity (against *S. epidermidis*).

Sodium chloride concentration (g/L)	ERI-1		ERI-3		ERI-26	
	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)	Dry cell weight (g/L)	Antimicrobial activity (mm)
4	1.85	17	1.93	18	1.93	18
8	3.21	18	2.84	18	3.71	21
12	4.68	23	3.64	24	3.53	22
16	3.43	20	2.94	20	2.14	22
20	1.91	18	1.54	19	1.33	21

Results were analyzed in triplicates.

Effect of sodium chloride on growth and antimicrobial activity

Effect of different concentration of sodium chloride on growth and antibacterial activity was studied. Increase in concentration of sodium chloride in the medium influenced the growth and antimicrobial compound production (Table 8). A concentration of 12 g/L was good for growth as well as antimicrobial activity for the strains. There was a decline in growth and antimicrobial activity after 20 g/L.

Optimized media on antimicrobial activity

Optimized media showed good activity as compared to normal fermentation media. Secondary metabolites from strain ERI-1 showed good antimicrobial activity in optimized media (M-4 media containing 12 g/L glucose, 1.2 g/L ammonium nitrate, 12 g/L sodium chloride, pH 7.0, temperature 30°C and incubation time 72 h). It was 1.5 times more than the normal media (Table 9). ERI-3 cultivated in M-8 media containing 12 g/L glucose, 1.2 g/L sodium nitrate, 12 g/L sodium chloride, pH 7.5, temperature 30°C and incubation time 72 h revealed good production of antimicrobial metabolite in the broth. M-4 media components with 12 g/L of glucose, 1.2 g/L ammonium nitrate, 12 g/L sodium chloride, pH 7.0, temperature 30°C and incubation time 72 h influenced ERI-26 for better production of antimicrobial metabolites.

Effect of fermentation media on antifungal activity

Fermentation media were also checked against fungal pathogens. It was observed that strain ERI-26 greatly suppressed the growth of all the chosen fungal strains as compared to strain ERI-1 and ERI-3 (Table 9). Among all the fungi, *A. niger* and *C. lunata* showed significant inhibition.

DISCUSSION

Cultivation of different complex media signalized the ability for secondary metabolite production and the influence of inoculum stage conditions. Arasu et al. (2008) reported that antagonistic properties are largely influenced by the quality of the medium. Eight different fermentation media were used for the selection of best antimicrobial metabolite production. Medium-4 (modified nutrient glucose agar media) was chosen as the best base for the production and antimicrobial metabolite production for ERI-1 and ERI-26. ERI-3 cultured in M-8 was the best source for antimicrobial metabolite production. Our results indicated that MNGA was the good base for antimicrobial metabolite production; however Augustine et al. (2005) reported that starch casein medium was found to be good base for antifungal metabolite production.

Optimizations of fermentation conditions are necessary to improve secondary metabolite formation. Dissolved oxygen tension was identified to influence the productivity

Table 9. Antimicrobial activity of optimized fermentation medium.

Strain	Antibacterial activity (Inhibition zones in mm)											Anfungal activity (Inhibition zones in mm)										
	B.s	***S.a***	***S.e***	***E.f***	***E.c***	***P.a***	***K.p***	***X.sp***	***E.sp***	***S.t***	***V.f***	***P.v***	***T.m***	***E.f***	***T.s***	***C.l***	***A.n***	***B.c***	**T.r 296**	**T.r 57**	**Scro**	**C.a**
ERI-1	22	18	18	19	15	16	23	18	15	15	15	10	-	-	-	-	-	-	-	-	-	-
ERI-3	22	20	21	12	11	10	12	15	10	12	10	13	-	-	-	-	-	-	-	-	-	-
ERI-26	21	23	20	15	13	12	10	11	11	11	10	11	15	15	10	20	25	10	15	18	10	18

- ; No activity, ERI- Entomology Research Institute. *B.s- B. subtilis*; *S.a- S. aureus ; S.e- S. epidermidis; E.f- E. faecalis; E.c - E. coli*; *P.a- P. aeruginosa; K.p - K. pneumoniae; X.*sp - *Xanthomonas* sp.; *E.*sp- *Erwinia; S.t- S. typhi*; *V.f- V. fischer*i; *P.v- P. Vulgaris; T.m- T. mentographytes*; *E.f- Epidermophyton floccosum*; *T.s- T. simii; C.l- Curvularia lunata*; *A.n- Aspergillus niger*; *B.c- Botrytis cinerea; T.r- Trichophyton rubrum*; *Scro- Scropulariopsis* sp.; *C.a- Candida albicans*. Results were analyzed in triplicates. Among the fungus, only *Candida albicans* was used in the initial antimicrobial screening such as effect of different medium, different temperature, different pH and different incubation time, respectively.

of several processes concerning bioactive compound (Ya-Jie et al., 2009). Possibility to enlarge secondary metabolite formation with high oxygen levels is a strong dependency of oxygen with the enzymatic reactions of product formation. Kim et al. (2000) reported a 7-fold enhancement of kasugamycin production by pH shock in batch culture of *Streptomyces kasugaensis*. It is well known that environmental signals, including pH shock, can stimulate and promote the biosynthesis of secondary metabolites. Kim et al. (2007) reported that when the pH was maintained at 5, production of geldanamycin was increased from 414 to 768 mg/L. Nadia et al. (2004) studied the effects of temperature, pH, incubation period, some media and different nitrogen and carbon sources for the production of antimicrobial metabolite production. Temperature of 35°C and pH 8 were the best for growth and antimicrobial agent production and 14 to 15 days of incubation was found to be the best for maximum growth and antimicrobial activity, respectively, in the medium BG-11.

We found that medium with glucose and fructose showed maximum antimicrobial metabolite production and cell growth. Ammonium nitrate and sodium nitrate were found to be the best nitrogen source for the antimicrobial metabolite production. They influence the growth, as well as the antimicrobial metabolite production of the actinomycetes strains. Nadia et al. (2004) reported that leucine was the best nitrogen source for antimicrobial activity, while maximum antimicrobial activity was introduced by using the carbon sources, citrate and acetate. Our results indicated that more antimicrobial activity was found when the carbon source is glucose in combination with the nitrogen source ammonium nitrate. The effects of nitrogen sources on streptolydigin production and distribution of secondary metabolites from *Streptomyces lydicus* were investigated in shake flask level (Liangzhi et al., 2007). When soybean meal was used as the source of nitrogen, three analogues of streptolydigin were detected. Among the nitrogen sources glutamic acid was most favorable for the formation of streptolydigin (Liangzhi et al., 2007).

ERI-1, ERI-3 and ERI-26 were able to grow in all the tested carbon sources. However maximum growth and antimicrobial activity was obtained in medium supplemented with glucose followed by fructose. Fermentation medium supplemented with glucose enhanced the growth and antimicrobial metabolite synthesis. The highest activity was obtained in media containing ammonium nitrate as a nitrogen source, followed by sodium nitrate and potassium nitrate. Strains cultivated at 30°C were optimum for good growth and antimicrobial metabolite production. The results also indicated an incubation time of 96 h as optimal. Cruz et al. (1999) reported that the production of antibiotic by *S. griseocarneus* was increased by glucose. Gupte and Kulkarni reported that three independent variables, namely concentration of carbon source (glucose), nitrogen source (soybean meal) and temperature of incubation, were found to be the most important for the production of antimicrobial metabolites (Gupte and Kulkarni, 2000). In general, *Streptomyces* sp. grew best in media containing carbon and nitrogen sources, including chitin, starch, glycerol, arginin, asparagine, casein and nitrate (Locci, 1989).

Results of the present work indicated that the selected actinomycetes possess antibacterial and antifungal properties in liquid broth. This explains that the antimicrobial metabolites are extra cellular in nature. Conditions such as pH, temperature and incubation period were optimized. Best me-dium for growth and antimicrobial metabolite production were selected on the basis of antimicrobial activity. Modified nutrient medium with glucose as a carbon source was found to be good base for fermentation. Best nitrogen source was selected based on the growth and antimicrobial activity. From

the present investigations, it was clear that ERI-1, ERI-3 and ERI-26 were found to produce extra cellular antimicrobial metabolites.

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